

## Journal of the Hellenic Veterinary Medical Society

Vol 73, No 2 (2022)



### Role of choline in ruminant nutrition: a detailed review

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doi: [10.12681/jhvms.26914](https://doi.org/10.12681/jhvms.26914)

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### To cite this article:

YAMAN FIRINCIOGLU, S. Y. F., & Irshad, N. (2022). Role of choline in ruminant nutrition: a detailed review. *Journal of the Hellenic Veterinary Medical Society*, 73(2), 3921–3928. <https://doi.org/10.12681/jhvms.26914>

## Supplementation of rumen-protective-choline and its role in dairy nutrition

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**ABSTRACT:** Recent studies designate feed additives, such as rumen-protected choline (RPC), fortified health and increased milk yield in ruminants. Choline is an endogenous compound, its deficiency leads to negative implications on the physiology and productivity of ruminants. Endogenous synthesis through the phosphatidylethanolamine N-methyltransferase (PEMT) pathway constitutes an unstable source of choline. Choline assimilation is affected by administered dose, lactation stage, and basal diet composition. High occurrence of fatty liver disease during lactation and especially in the periparturient period is observed in dairy cows and to alleviate such incident, high supply of choline is necessary. This supply is known to decrease the chances of hepatic fatty infiltration and upregulate the expression of genes involved in the transport of VLDL (very low-density lipoprotein). RPC is usually provided as choline chloride and is contained inside a shield of the fatty acid matrix. Therefore, choline contribution into important mechanisms, via metabolite of betaine is ensured by its provision in a rumen-protected form in the ration of dairy cows. These important mechanisms are sparing of a methyl group (-CH<sub>3</sub>), supporting of animal metabolism and remethylation of homocysteine. It is noteworthy that rumen-protected choline positively affects the milk yield of animals during the lactation period especially when supplementation started before calving and continues throughout the early lactation. But the discrepancy of commercial products of rumen-protected choline regarding their degradability in the rumen and choline content is one of the big problems yet to be resolved.

**Keywords:** Dairy cattle; choline; ruminants; lactation; methionine.

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*Date of initial submission: 29-04-2021*  
*Date of acceptance: 21-10-2021*

## INTRODUCTION

Modern methods of feed formulation as well as the provided nutrient requirements based on the needs and the production phase of the animal contribute greatly to the health and production of ruminants. Excessive or unstable quantity of nutrients may lead to an unbalanced diet that is further related to illness, increases environmental pollution as well as decreases dairy performance. Choline is complex like a vitamin, its metabolism thoroughly linked with the metabolism of methionine and vitamin B12 and the deficiency of choline is devastating in ruminants (Hollenbeck, 2012). Recent studies suggested that feed additives, such as RPC, can improve health status, milk yield, and welfare in ruminants. Erdman et al. (1984) reported that choline is susceptible to fast ruminal degradation, and subsequently the quantities available for absorption are limited. More than 80% of choline chloride ( $C_5H_{14}ClNO$ ) supplements are widely degraded by the microbial population of the rumen and only a small amount is available for absorption. As a result, choline provision as rumen-protected could improve its availability. RPC is typically provided as choline chloride by a shield of fatty acid matrix and the microbes present in the rumen cannot digest choline chloride (Luciano et al. 2020). In this way, the breakdown of fatty acids occurs in the small intestine by the digestive enzymes and free choline chloride is ready for absorption. In this way, RPC can be beneficial for dairy cows. When choline is limited in dairy cattle diets it directly leads to methionine deficiency and contrariwise. Zeisel (2000) reported that choline is an indispensable nutrient for ruminants when they are offered diets that have a serious deficiency of methionine and folate. Totally 28% of absorbable methionine (Met) is used for the synthesis of choline in lactating goats (Emmanuel and Kennelly 1984). The application of RPC product "Bio-Choline" (with conjugates of choline) could serve as an alternative and it is a natural product that offers high bioavailability and could replace choline chloride, leading to improvement of growth in lambs (Godinez-Cruz et al., 2015) and milk yield in ewes (Crosby et al., 2017). Elsayy et al. (2014) reported that choline also has antioxidant properties and it is especially beneficial in transition cows as it results in the decrease of free radicals' production that can harm liver cells. In this review, we will discuss choline functions, its mechanism of action, the importance of choline in periods of transition and lactation in dairy cows, the role of RPC and, the significance of bio-choline in ruminants.

## SOURCES OF DIETARY CHOLINE

Various forms of choline (i.e., acetylcholine, choline-containing phospholipids, and free choline) are intrinsically produced by the animal or are received by plant tissues. The maximum amount of choline is available as phosphatidylcholine i.e., lecithin in feed elements and crude unrefined sources of fats, while choline chloride that contains 87% choline is frequently added as a substitute to a natural source in process of compound feeding (Pinotti et al., 2002).

## CHOLINE METABOLISM IN RUMINANTS

The discovery of choline in different forms is pretty normal both in animals and humans. Different kinds of water-soluble metabolites of choline including acetylcholine (an imperative neurotransmitter which plays a vital role in the neuromuscular system), betaine (an oxidative intermediary category of choline which provides a  $-CH_3$  for the conversion into Met and glycerophosphocholine from homocysteine) as reported by Zeisel, (2000) and Artegoitia et al., (2014). There are also various structural constituents of mammalian membranes, such as lysophosphatidylcholine (LPC), sphingomyelin (SM), and phosphatidylcholine (PC) that are lipid-soluble choline-containing metabolites (Baldi A and Pinotti L 2006). For the export of triacylglycerol from the liver, a particular mechanism is a synthesis of very-low-density lipoprotein (VLDL), which is usually implemented by PC (Luciano et al., 2020). The PEMT pathway of choline and its metabolites is shown in Figure 1.

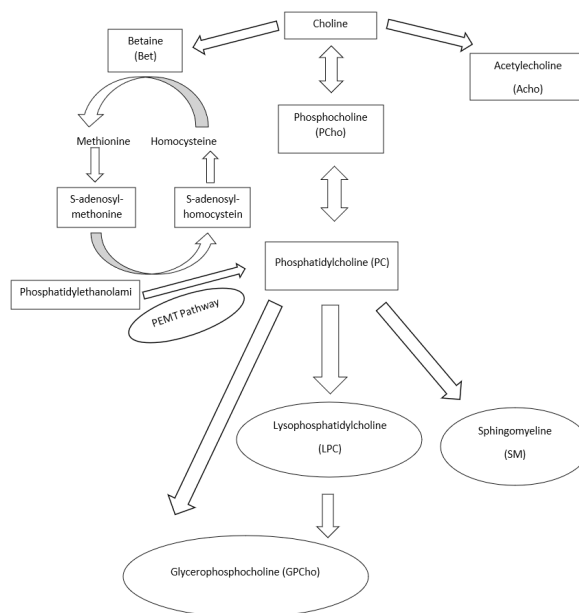


Figure 1. Metabolism of choline and its metabolites

The compounds shown in boxes were assayed in milk and plasma. Phosphocholine and phosphatidylcholine are formed from choline via the cytidine diphosphate (CDP) choline pathway. The formation of betaine from choline is irreversible. Betaine when oxidized will provide a methyl group to homocysteine to form methionine. Methionine is converted to S-adenosylmethionine, which is an important methyl donor. Phosphatidylcholine can be formed endogenously by methylating phosphatidylethanolamine in a three-step process involving S-adenosylmethionine via the phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway.

Apart from dietary choline, choline is produced through the pathway of PMET (endogenic biogenesis that primarily takes place in the liver) (Steven et al., 2003). Supplementation of choline for maintenance of natural organ function by the pathway of PEMT indicates that only one way of choline production is not sufficient (Zeisel, 2006). More than 80% of choline is degraded by ruminal bacteria and thus endogenous synthesis through this PEMT pathway constitutes an unstable source of choline (Vance, 2013).

#### ASSESSMENT OF CHOLINE STATUS

Choline (in addition to vitamin B12 and folates) plays a significant role in the metabolism of 1-carbon (a multifaceted tract of related biochemical responses involved in the transmission of individual carbons). Therefore, a clear position of choline, vitamin B12, and folates need to be determined in plasma (Mason 2003). This method will allow the development of combined measurement of all the important nutrients tangled in the metabolism of 1-carbon and could contribute to an inclusive suggestion about the capacity of methylation in living organisms. Pinotti et al., (2004) observed that providing 50 gram of RPC (which offers 20 g of choline chloride to the dairy cows in their transition period) increased levels of plasma choline-holding phospholipids (127 vs. 171-milligram dL<sup>-1</sup>, *P* equal to 0.08 and folates i.e., 6.74 vs. 9.68 nanogram mL<sup>-1</sup>, *P* lesser than 0.05) during the final phase of the transition period. Thus, it is indicated that the RP form of choline is operational both in enhancing the metabolism of -CH<sub>3</sub> and increasing the availability of choline. A beneficial tool to access the status of choline in ruminants is to measure the choline content of milk. These reports also presented that the normal level of choline in cow milk is about two grams per day. The total secretion of choline in the milk of dairy cows was 3.81 gram per day and this amount

is only presented in cows administered with RPC in contrast to controls which showed the value of 2.53 gram/day. On the other hand, concentrations of choline were 138.5 milligrams per kilogram, in milk collected from RPC supplemented animals that showed an increase of 39% in contrast to controls (99.66 milligrams per kg). The retrieval of administered choline in milk was assessed to be 6% based on the upsurge of choline in treated cows. This investigation delivers further indication that in dairy cows' milk choline is highly sensitive to post-ruminal choline supply. Therefore supplements of choline should be provided in RP-form to work more effectively. To guard an element against degradation in the rumen a process called microencapsulation is widely used (Shahsavari et al., 2016). Microcapsules can be formed and allow precise discharge of the supplement at an anticipated site in the intestine, thus refining its efficiency, safeguarding optimal dose as well as widening the applying time of the feed and its components (Yang et al., 2019). It is necessary to know that the concentration of choline is different according to fat content, lactation stage, breed, and method of assay (Bjerre-Harpøth et al., 2012).

#### CHOLINE AND ITS LIMITING FACTORS

Dosage, manner of administration form, stage of lactation, as well as basal diet composition are some of the factors which limit the bioavailability of choline in dairy cows.

##### Administration dose:

By administering 12 to 20 grams per day of choline (i.e. RPC form) a fair milk yield can be achieved. McDowell (1989) suggested that such doses may seem too high, but this kind of dose requires more hydro-soluble vitamins i.e., gram vs. milligram. Mostly, RPC was provided as a topdressing which shows that mode of administration plays a significant role (Hartwell et al. 2000; Scheer et al. 2002; Pinotti et al. 2003; Piepenbrink and Overton 2003). Pinotti et al., 2003, 2004) performed two different studies and later compared the results. In the first study (2003), they administered RPC at a dose rate of 20 gram per day (as a top-dress) which raise the milk yield of RPC-supplemented cows to 2.9 kilograms per day throughout the first 30 days of lactation. In the second study (2004) same dose rate was administered (20gram per day) during the first month of lactation. However, during this study, RPC was incorporated into the compound feed tended to change milk production (27.05 vs.

**Table 1.** Level of choline used in form of RPC in different years

Publications	Level of choline in form of RPC	Year
Erdman and Sharma	0-45g/day	1991
Erdman	0-35g/day	1994
Bindel et al	0-15g/day	1998
Pinotti et al	0 to 20g/day	2003
Pinotti et al.	0 to 50g/day	2004
Baldi and Pinotti	0 to 60g/day	2006
Lima et al	15g/day	2007
Pires and Grummer	15g/day	2008
Chung et al	25 to 50g/day	2009
Sales et al	6 to 50g/day	2010
Goselink et al	14.4 g /day	2012
Huawei et al	0.0 to 0.75% of DM	2015
Zhou et al	60g/day	2016
De Veth et al	12 to 25g/day	2016
Martínez-Aispuro et al	0 to 9 g/kg DM	2019
Kawas et al	0 to 0.75g/day	2020
Ahmadzadeh-Gavahan et al	6g/day	2021

25.10 kilograms per day in RPC cows) and control. This inconsistency showed the diverse manner of administration of the RPC. The values of the average intake of choline before and after the parturition time were 92% and 84%. Hence in the second study the total choline intake was lower than the first one due to alteration in its administration way.

### Lactation stage

The highest demand for choline is at the lactation stage as choline and its related nutrients like Met, vitamin B9 and vitamin B12 are present in lesser quantities (Preynat et al., 2009). Metabolic changes occur from late gestation to early lactation and during these critical times choline is particularly beneficial for animal health (Piepenbrink and Overton 2000). An interesting connection between time and treatment for both NEFA (plasma non-esterified fatty acids concentrations) and plasma NEFA was stated by Pinotti et al. (2003). This interaction showed that NEFA level increased due to fat mobilization at the time of parturition in animals supplemented with RPC.

### Basal diet composition

The significance of diet and feed composition in choline administration is very high as they affect the supply of protein and the availability of other nutrients that directly affect choline supplementation. For instance, administrating 12 grams of RPC per day (starting 28 days ahead calving) improved milk production. This RPC was certainly administrated with

the compound feed. This kind of supply during the first 8 weeks of lactation worked well when provided with a lesser lower amount of rumen undegradable protein (RUP), reported by Hartwell et al. (2000). De Veth et al (2016) experimented on 5 late lactation Holstein cows using two different treatment sources. In the first case, 12 and 25 grams of choline were given each day in RPC form with a control group. In the second case, 12.5 and 25 grams of choline were offered via abomasal delivery of choline (ADC). The results showed that by increasing the dosage via abomasal delivery of choline the entrance chances of free choline raised meanwhile, this free supply increased some metabolites and all of them can be worked as biomarkers to check the bioavailability of choline.

## EFFECTS OF CHOLINE

### SUPPLEMENTATION ON DAIRY ANIMALS

Even though the ideal doses of choline for dairy cows are not properly identified yet, higher availability of choline in form of RPC could have a significant effect on milk yield as stated by Piepenbrink and Overton (2003). Table 1 shows several publications that used different levels of choline chloride as RPC in various studies.

Pirestani and Aghakhan (2018) conducted a number of experiments and suggested that the administration of combined dose of choline and L-carnitine improved the reproduction to a significant level in dairy cows.

### Fatty liver syndrome

In dairy animals, choline is widely degraded in the rumen. Zeisel (2000) reported that choline plays the role of a significant nutrient for the health of ruminants especially when it is offered with methionine and folates deficient diet. The main form of choline phospholipids is Phosphatidylcholine (makes up more than 50% of phospholipids in mammalian cell membranes). It is also an important constituent of VLDL and cannot be replaced with other phospholipids. The deficiency of choline may decrease the formation of VLDL and leads to fatty liver (the basic reason is the limited exportation of triglycerides from the liver). Supplementation of choline significantly increased VLDL secretions from rat liver, as suggested by Zeisel (1993); but also Met supplementation upsurges secretions of VLDL in calves (Auboiron et al., 1994). During early lactation, the dietary supply of choline and methionine can reduce the incidence of fatty liver in dairy cattle. Zhou et al., (2016) performed methionine and choline treatments in an experiment with 48 multiparous Holstein cows. The results showed that both Met, and Choline have positive effects on liver functions and immunity. Overall, methionine-supplemented cows displayed better results as compared to choline-supplemented ones.

### The oxidation of NEFA

There are various physiological problems that cows are facing during their transition period. The low dry matter intake (DMI), huge demand for good quality nutrients, hypocalcaemia, fatty liver signs, negative metabolizable protein balance, the progress of ketosis, and clinical mastitis are some of them (Overton and Waldron 2004; Esposito et al. 2014; Itle et al. 2015; Sun et al. 2016). To defeat the negative balance of energy in transition cows the amount of esterified fatty acids (NEFA) is enhanced by stress and hormonal variations during this period (Shahsavari et al., 2016). To generate energy, the oxidation of NEFA occurs in the liver and TG (triglyceride) is produced that could be used for VLDL (Figure 2).

Lipolysis of adipose tissue grounds the discharge of NEFA into the blood. These non-esterified fatty acid amines are either oxidized to ketones (to provide ATP for liver metabolism) or esterified to TG. Ketones become unconfined into the blood as well as additionally oxidized by muscle. On the other hand, liver triglyceride can be deposited as droplets (fatty liver) or packaged into VLDL and transferred into the blood. The synthesis of the apo-lipoprotein com-

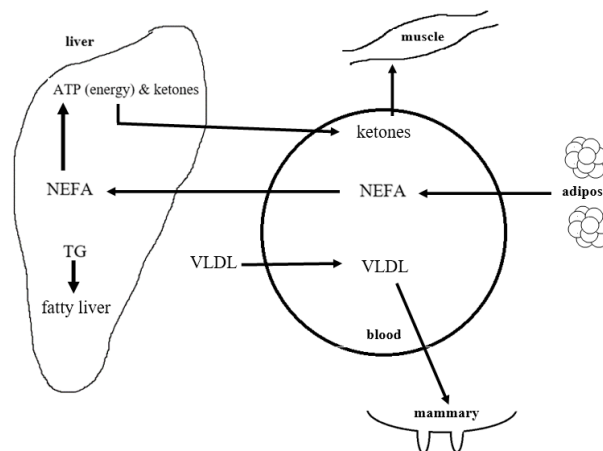


Figure 2. Mechanism of choline in the dairy cow

ponents (of VLDL) may be affected by choline as it helps to increase triglyceride export from the liver.

However, some of the esterified fatty acids are moderately oxidized in the liver and therefore the transfer of triglyceride through very-low-density lipoprotein as well as ketone content in the blood is reduced. Besides, the ratio with peroxidation lipids is increased in early lactation. Serum  $\alpha$ -tocopherol levels are reduced, and higher oxidative stress rates are triggered that impair the performance of dairy cows, as indicated by LeBlanc et al., (2004). Sun et al., (2016) suggested that supplementation of RPC and RPM (rumen-protected Met) alleviate oxidative stress as well as improve immune competence during the transition period. Microbiotic organisms present in the rumen of dairy animals degrades the choline fastly and the remaining amount of choline cannot fulfil the important role of maintenance. Therefore, choline must be provided in the rumen-protected-form in the ration of dairy cows to contribute in the important mechanisms, like sparing of a methyl group (-CH<sub>3</sub>), supporting of animal metabolism, and remethylation of homocysteine via metabolite of betaine (Esposito et al., 2014; Itle et al., 2015).

### Milk fat synthesis

Choline plays a very important role in milk yield and synthesis of milk fats. Many experiments showed that supplementation of choline in RPC form can increase the production up to a significant level. Elek et al., (2008) performed a research at thirty-two Holstein cows and divided them into two groups (RPC treated and control). Throughout the periparturient period, the goals were to consider the effects of milk production, milk fat contents, and milk yield. The results showed

a visible increase in milk yield (4.4 kg higher for RPC treated group). Four percent fat corrected milk also increased by 2.5 kilograms per day. Although fat contents of milk remained the same, an increase in fat yield was observed (0.10kg/d). The most recent examination suggested that supplementation of dietary choline during the period of transition increased milk yield as well as ECM in multiparous dairy cows (Arshad et al., 2020).

### **Growth performance**

RPC plays a significant character in the growth of ruminants as it is considered as the most commonly assessed source of choline for small ruminants (Bryant et al., 1999, Godinez-Cruz et al., 2015, Tsiplakou et al., 2016, Habeeb et al., 2017). Supplementation of choline in form of RPC effects the average weight gain in ruminants. A study where 24 Dorper × Hu lambs were fed diets incorporated with 0, 0.25, 0.50, and 0.75 percent of rumen-protected choline for 60 days, revealed that supplementation of 0.25 percent of RPC increased the average daily gain of lambs however, these treatments had no important effect on feed intake (Huawei et al., 2015). Meanwhile higher doses like 0.75 percent showed harmful effects on live weight gain. De Veth et al., (2016) observed that just up to 61% of choline chloride is absorbed after reaching the duodenum. In one recent experiment, forty intact male Saint Croix lambs were used and randomly assigned to 4 treatments 0, 250, 500, and 750-milligram Rumen-Protected-Choline/Day. When RPC was offered with a high roughage grain diet, the results showed no significant effect on carcass quality and productive performance (Kawas et al., 2020). The collective supplementation of RPC, propylene glycol (PG), and monensin sodium (MS) in pregnant Ghezel ewes, raised the concentrations of glucose, cholesterol, triglycerol, insulin, albumin as well as total protein content. Ewes also lose less weight and improve BCS during this period (Ahmadzadeh-Gavahan et al., 2021)

### **BIO-CHOLINE A RECENT APPROACH**

A new product containing microencapsulated choline chloride has been prepared by researches and by commercializing this kind of product, about 85% of choline usually wasted due to rumen degradability can be retained. These kinds of commercially available choline products can be supplemented directly in compound feed or added as a top dress. A substitute to swap is RPC product, Bio-Choline (a feed flora

accumulative with conjugates of choline) that has a confrontation with degradation of the rumen, as it is established by falling out with lamb in growing stage (Godinez-Cruz et al., 2015). This product of choline also showed an important effect on production of ewe milk (Crosby et al., 2017). Martínez-Aispuro et al., (2019) carried an experiment with forty male Hampshire × Suffolk lambs to evaluate the effect of herbal Bio-choline. Amount of 0, 3, 6, and 9 g/kg DM of Bio-choline were included in lamb diets for fifty-six days continuously. The results showed a positive change in daily weight gain, feed conversion, blood phosphatidylcholine, level of glucose in the blood, and final weight gain. Thus, it can be stated that such products of choline can be considered as a good source to fulfil the daily choline requirements in ruminants. However, the difference of commercial products of rumen-protected choline in their rumen degradability and choline content is one of the big problems yet to be resolved (Brusemeister and Sudekum 2006).

### **CONCLUSION**

In conclusion, the dietary supplementation of choline seems to be insufficient to raise the production of milk in dairy animals particularly at the start of a period of lactation. Choline production is affected by administered dose, lactation stage, and basal diet composition. Precisely concerning lactation, it is important to note that rumen-protected choline positively affects the performance of animals during the lactation period especially, when supplementation started before calving and continues throughout the early lactation. It is better to assume that animals are having a huge demand for choline during pregnancy and lactation periods and the availability of other choline-associated nutrients is also low. Supplementation of dietary choline plays a major role in growth performance of ruminants particularly in finishing cattle and its availability also has a huge impact on milk fat synthesis and energy corrected milk (ECM). Absorption of methionine also plays an important role in the production and limitation of choline in dairy cattle. The deficiency of choline may decrease the formation of VLDL and cause fatty liver in ruminants. Therefore, enhancing the dietary supply of choline as well as methionine can lower the frequency of this disease during the phase of early lactation. The most recent examinations suggested that supplementation of dietary choline during the period of transition increased milk yield as well as ECM in multiparous dairy cows. Addition of RPM and RPC alleviates oxidative stress

as well as its antioxidant activities on immune organs during the transition period. Subsequently, the available data about the role of RPC is still insufficient but it can be suggested that administration of RPC plays a significantly positive role in ruminants during the transition period. The substitutes to swap the RPC are present in the market, which can confront with degradation of the rumen and about 85% of choline that usually wastes due to rumen degradability can be retained. These kinds of commercially available choline products can be supplemented directly in compound feed or added as a top dress. A feed floral-herbal prod-

uct called Bio-choline is getting popular in small ruminants these days as it is working quite efficiently by transferring choline smoothly and saving it from ruminal degradation. Despite all this, there is still a big gap between available data regarding the correct administration of choline and its concerning products. Meanwhile, the reason for the weak performance of animals during some experiments also needs to be discussed further.

## CONFLICT OF INTEREST

None declared.

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